CD36 receptors with a primary Fab fragment and a Cy3-conjugated secondary Fab fragment or with quantum dots, and imaged the dorsal surface of macrophages using live-cell epifluorescence microscopy. To track the imaged CD36 receptors, we developed a single-particle tracking algorithm that detects particles with sub-pixel localization and follows dense particle fields, capturing particle association and dissociation events and recovering trajectory interruptions resulting from temporary particle disappearance (Jaqaman Nat. Methods 2008). We found that, at rest, surface-bound CD36 receptors existed as discrete multimers that contained up to six monomers. A subpopulation of the detected CD36 multimers (~30%) moved along linear tracks that radiated from the perinuclear region. The movement required both the acto-myosin network and microtubules. Temporal multi-scale analysis of receptor frame-to-frame displacement and run-time toward and away from the perinuclear region using sampling frequencies of 10-125 Hz revealed that the linear motion of CD36 multimers was due to constrained diffusion within cytoskeleton-mediated linear corridors in the membrane. Importantly, the dimensionality reduction resulting from motion within corridors increased the probability of CD36 aggregation two-fold. Cytoskeleton perturbations that inhibited the linear motion of CD36 inhibited the uptake of its ligands and the phosphorylation resulting from CD36 cross-linking. These data provide the first direct demonstration of the functional requirements of cortical cytoskeletal structures in the regulation of receptor activation via the spatial organization of receptor motion and aggregation at the level of the plasma membrane.

1435-Pos Board B279

Plasma Membrane Topology and Membrane Models

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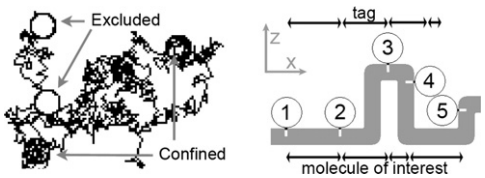
Single particle tracks (SPTs) can be followed over the plasma membrane with high spatial and temporal resolution. The interpretation of SPTs, combined with Monte Carlo simulations, form the basis of complex membrane models. Most interpretations of SPTs rest on the convenient assumption that cell membranes are flat. However simulations of simple diffusion over surfaces that include pillars, when the locations are known in X and Y but movement is possible in Z, substantially reduce the apparent rate of diffusion and produce tracks that could be interpreted as transient anchorage and confinement (Figure, left).
In addition SPTs usually follow a tag attached to the molecule of interest, not the molecule itself. When the molecule is confined to the plasma membrane, with the tag in the extracellular fluid and the surface is flat, they may be coincident. However gradients produce a variable offset between the molecule and its tag, misreporting the actual movement (Figure, right).
Plasma membranes are not flat and projections or depressions produce the appearance of anomalous subdiffusion. We suggest that complex explanations, transient anchorage or barriers, should only be considered once more obvious ones have been disproved.



1436-Pos Board B280

Lateral Organization in Simulated Four-Component Non-equilibrium Model Membranes

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Lateral organization in biomembranes plays a major role in membrane topology, and is thus implicated in many basic functions of biomembranes such as endocytosis and signal transduction. In this study, non-equilibrium Monte Carlo simulations are used to investigate two related scenarios: 1. the effect of a rigid distribution of proteins on the lateral organization of lipids in a biomembrane, and 2. the degree to which lipid interactions influence the lateral organization of membrane-associated proteins that are free to translate laterally. Our model includes generic saturated and unsaturated lipids, proteins, and cholesterol, and is driven out of equilibrium through simulated endo- and exo-cytosis events. By varying the temperature, the protein mole fraction, and the interaction strengths, we examine the conditions under which various types of lateral organization occur. Simulation results are analyzed with pair-correlation functions and the Ripley K-test. We compare results from simulations of the two scenarios above and from simulations of biomembranes lacking protein.

1437-Pos Board B281

Growth Cones As Sensing, Amplifying And Filtering Modules

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Sensitivity to weak directional signals is a striking feature of chemotactic systems. In eukaryotic cells, it is often attributed to spatial amplification in the detection of the gradient of guidance cues due to an asymmetric distribution of signaling molecules of the signaling pathway. By combining single-quantum dot imaging with a guidance assay, we probed the dynamics of GABAA receptors (GABAARs) in nerve growth cones. In presence of a GABA gradient, we observed a lateral redistribution of the receptors towards the GABA source. This effect was both reversible and specific of GABA signals. Its functional implication was established by calcium imaging which showed that the redistribution was accompanied by an enhanced asymmetry of the calcium response. Furthermore, single quantum-dot tracking of GABAARs revealed a ''conveyor-belt'' type of motion in which receptors randomly alternated between periods of free diffusion and of microtubule-dependent directed movement.
We therefore propose a model in which an asymmetric activation by the signaling gradient leads to oriented growth of the MTs which, in turn, contributes to an asymmetric distribution of the receptors and amplification in gradient sensing. This simple model for the formation of polarity at the cell membrane is supported by numerical simulations that describe with minimal hypothesis the results of our experiments. These simulations also provide predictions on the dependence of the formation of polarity as a function of gradient parameters. We will finally show our current effort to place neurons in microfluidic devices, to generate controlled gradients and to characterize the growth cone as a sensing, amplifying and filtering module.

1438-Pos Board B282

Imaging Of The Diffusion Of Individual Band 3 Molecules On Whole Erythrocytes From Patients With Hereditary Hemolytic Disorders

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The plasma membrane of the human erythrocyte is a composite structure that consists of a fluid lipid bilayer (including membrane-embedded and membrane-associated proteins) and a sub-surface scaffolding consisting of a six-fold network of the tetrameric flexible structural protein spectrin. The spectrin network is pinned to the fluid bilayer through a series of transmembrane and membrane associated proteins both at the six-fold junctions and near the mid-points along the spectrin tetramers between junctions. Common to both of these pinning points is the presence of the transmembrane anion exchange protein band 3 (AE1). Band 3 ties the six-fold junction to the bilayer through interaction with the protein adducin and ties the midpoint to the bilayer through interaction with ankyrin. Much of the remarkable mechanical characteristics of the red cell have been attributed to this membrane architecture. Further, red cells become fragile in pathologies known to disrupt the spectrin network or its pinning points to the membrane. To assess changes in the structure of the plasma membrane of pathologic red cells at the single molecule level, the mobility of individual band 3 molecules was observed on normal red cells as well as those from patients with several types of hereditary diseases including spherocytosis, elliptocytosis, and pyropokilocytosis. Specifically, individual band 3 molecules on whole red cells were labeled by quantum dots through the band 3 inhibitor 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS). We will present data on the mobility of band 3 in each of these cell types recorded at video imaging rates of 120 fps. The observation that membrane pathologies can be distinguished by the mobility of individual membrane molecules suggests that single particle tracking might constitute a useful tool for characterizing the ''health'' of a membrane.

1439-Pos Board B283

Dynamics Of Folate/Folate Receptor Complexes In KB Cells Observed Through Single Molecule Fluorescence Imaging

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Folate (vitamin B_9) is essential in the synthesis of nucleotide bases and amino acids. Cellular uptake (endocytosis) of folate is mediated by the membrane bound folate receptor (FR), a glycosylphosphatidylinositol-anchored protein. FR has been found to be up-regulated and/or redistributed in the plasma

membrane of specific types of carcinoma cells. Specifically, the over-expression in certain epithelial carcinoma cells can be as high as a thousand fold and thus FR provides an important possible target in efficient drug therapy design. In this study, we are probing the dynamics and interactions of folate/FR receptor complexes in the plasma membrane and through each part of its endocytotic cycle at the single molecule level. We are employing single molecule fluorescence microscopy to track individual fluorophore-labeled folate molecules bound to cell surface FR. We found an average diffusion constant of D = 2e-9 cm^2/s on live human KB carcinoma cells when imaged at 30 fps. Trajectories of individual particles showed temporary stopping or confined motion. This anomalous diffusive behavior was quantified against Monte Carlo simulations of randomly diffusing particles. The frequency and duration of confinement was compared as the overall concentration of folate was incrementally increased from 1 fM to the physiological level of 1nM . The effect on the frequency and duration of single FR confinement due to cholesterol depletion, actin stabilization and actin depolymerization will be presented.

1440-Pos Board B284

The Role of Cluster Size and Protein Spatial Pattern in the Immunological Synapse

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During antigen recognition by T cells, signaling molecules on the T cell engage ligands on the antigen-presenting cell and organize into spatially distinctive patterns collectively known as the immunological synapse (IS). The spatial arrangement of proteins into well-defined zones within the IS is known to regulate T cell activation and signal transduction. The mechanisms by which this complex organization arises remain unclear. Here we alter the clustering state of the T cell costimulatory molecule, LFA-1, either by direct antibody crosslinking or by crosslinking its ligand, ICAM-1, displayed on the supported bilayer. Changes in receptor clustering lead to progressively more central localization of LFA-1 until it colocalizes with T cell receptors (TCR) at the center. The number of LFA-1 molecules within the resulting clusters is obtained by fluorescence correlation spectroscopy. Our results demonstrate that cluster size is a critical parameter in determining protein spatial positioning in the IS. We discuss a sorting mechanism, based on frictional coupling to the cytoskeleton, which is consistent with these observations and is, in principle, extendable to all cell surface proteins in the synapse. Furthermore, by presenting patterns of immobilized ICAM-1 within a fluid bilayer displaying the TCR ligand, peptide-loaded MHC, we investigate the importance of LFA-1 ring formation to T cell function and signaling.

1441-Pos Board B285

Multispecies Single Molecule Imaging With Quantum Dots

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Quantum dots (Qdots) are fluorescent nanoparticles that have far superior signal intensity and signal stability compared to more conventional fluorescent molecules. We find that imaging with Qdots can easily be extended to the simultaneous visualization of up to four different molecular species at single molecule sensitivity and millisecond time integration. We find that this technique can easily be adapted towards studying the spatial and temporal nano-organization of various combinations of lipids and proteins in the cellular plasma membrane.

Intracellular Communication & Gap Junctions

1442-Pos Board B286

Structural And Functional Significance Of The N-terminus Of Cx26 Gap Junction Channels

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Investigations of gap junction channels have used different and often complementary experimental approaches to elucidate the gating mechanism of these intercellular conduits. However, no consensus gating model explaining the different phenomena of voltage and chemical gating has been formulated that is consistent with all mutagenesis, dye permeation, electrophysiology and electron microscopy results.

We made several N-terminal deletion constructs to assess channel structure/function. Shorter deletions such as del2-7 and del2-8 formed gap junctions when expressed in mammalian cells while longer constructs (del2-10 and 2-11) were either much less efficient or failed to form gap junctions. For our structure analysis, a full length connexin26 (Cx26M34A) and an N-terminal deletion mutant (Cx26M34Adel2-7) were over-expressed using Sf9 insect cells. Purified proteins were reconstituted into the lipid bilayers that formed 2D crystals. The 3D maps at 10Å resolution revealed that crystals obtained from both constructs were composed of three lipid bilayers with the channels forming a $p22_12_1$ lattice. The structure of Cx26M34A clearly showed a prominent density we refer to as a ''plug'', which resides in each hemichannel pore and contacts the innermost helices of surrounding subunits at the bottom of the vestibule (Oshima *et al.*, 2007). The 3D structure of Cx26M34Adel2-7 contained a reduced plug and a partially reduced density in the cytoplasmic domain that bridges the adjacent four helix bundles, suggesting that the N-terminus of Cx26 has an important role in forming a plug. Cx26M34Adel2-7 exhibited no electrical functionality. Cx26M34A channels showed little or no dye transfer and a dramatically reduced conductance, although the voltage gating characteristics of the residual conductance were normal. Physical blockage may be one of the gating mechanism of Cx26 channels, however, this may represent only one of multiple gating configurations.

1443-Pos Board B287

Effects Of Induced Post-ischemic Phosphorylation On Action Potential Propagation In Mouse Neonatal Cardiomyocytes

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Intracellular events triggered by protein phosphorylation and kinase translocation have been reported to help preventing larger cardiac tissue damage. Our objective was to determine if immediate changes in phosphorylation post-infarction, resembling clinical treatments could affect conduction velocity and further cardiac protection.

Cardiomyocytes from 0-2 day old mice pups were grown on 64 Micro-Electrode Arrays (MEAs; MES, Germany). The MEAs recorded action potential (AP) propagation produced by neonatal cardiomyocytes isolated and culture for 2 days. Myocytes were subjected to ischemia by placing a 13mm glass round cover-slip over the preparation for 45 minutes and recordings were made during and after cover-slip removal. After the ischemic event, random groups were treated with 300nM TPA to induce phosphorylation for one hour. TPA was removed and both groups were incubated for 24 hours. Following incubation cells were subjected to another ischemic event. Recordings were done after the ischemic event and again thirty minutes later. Conduction velocity (CV) was averaged from all electrodes.

Phosphorylation and kinase translocation has been known to protect cardiocytes during ischemic preconditioning. We now present conditions where phosphorylation can actually be detrimental if induced after an ischemic event. At the 24 hour point, the control group had recovered AP CV by $99 \pm 2\%$ (mean $\pm$ SE) compared to the TPA treated group which recovered to $56 \pm 8\%$. The TPA treated group then showed only an average of $14 \pm 7\%$ decrease in AP CV and AP amplitude upon administering the second ischemic event whereas the control group AP CV decreased by an average of $55\% \pm 9$. When induced early after an ischemic event, phosphorylation results protective to maintain CV but appears to become detrimental for cellular survival.

1444-Pos Board B288

Different Types of Cell-to-Cell Connections Mediated By Nanotubular Structures

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Communication between cells is crucial for proper functioning of multi-cellular organisms. The recently discovered membranous tubes, named tunnelling nanotubes (TNTs), that directly bridge neighbouring cells, may offer a very specific and effective way of intercellular transport and communication. Our experiments on RT4 and T24 urothelial cell lines show that TNTs can be